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PRINCIPAL INVESTIGATOR: Charlie Chen, Ph.D.

CONTRACTING ORGANIZATION: University of California
Los Angeles, California 90095-1406

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Charlie Chen, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of California
Los Angeles, California 90095-1406

E-MAIL:
charlie@math.ucla.edu

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Annual Summary Report

RE: Award Number DAMD17-99-1-9514

Awardee: Charlie D Chen

An Overview of My Past Year Performance

In my first year training that was supported by the DOD, I have been working on the identification of genes involved in the androgen independence progression of prostate cancer at the University of California, Los Angeles (UCLA), with Dr. Charles L Sawyers. This training not only guides me to a better understanding of prostate cancer progression and scientific research as a whole, but also reassures that I want to devote my career to the prostate cancer research.

During the course of this training period, I organized a joint meeting among four laboratories at UCLA who are studying the progression of prostate cancer. I regularly presented my experimental data in the laboratory meeting and the joint meeting. I attended seminar series such JCCC seminar, pharmacology department seminar. I reviewed papers that were submitted for publication in journals such as Molecular and Cellular Biology, Oncogene, Cancer Research, Cell Growth and Differentiation, and the Journal of Clinical Investigation. I became a reviewer for Cancer Research.

I have also accomplished a great deal in my scientific research. I successfully identified two genes, NF-kB and BRCA1, that may be important for prostate cancer progression. My results were presentated in 1999 in the 91st annual meeting of the American Association of Cancer Research (AACR) in San Francisco, California. I have also submitted an abstract for the 92nd annual meeting of the AACR.

The training further establishes my career goal as to be a scientist in prostate cancer research. I hope I can continue to have support from the DOD as I believe that I will continue to make significant contributions to the understanding of prostate cancer progression to androgen independence.

Enclosed please find the summary of my research progress, which contains two parts: Research Progress Part I, evidence implicating NF-kB in androgen-independent prostate cancer progression; Research Progress Part II, BRCA1 inhibits the transcription activity of androgen receptor by recruiting HDAC complex.

Research Progress Part I, Evidence Implicating NF-kB in Androgen-Independent Prostate Cancer Progression

Previously we showed that the mitogen-activated protein kinase kinase kinase 1 (MEKK1) activates the promoter and enhancer of prostate specific antigen (PSA), a marker for prostate cancer development and progression. Because we were unable to detect changes of phosphorylation of AR, we hypothesized that the transcription activity results from the activation of AP-1 and/or NF-KB, two notable MEKK1 downstream effectors involved in transcription regulation. To further characterize the mechanism of activation, we carried out reporter assays that read out each pathway independently by transient transfection.

The transcription activation of MEKK1 on the promoter and enhancer of PSA does not result from AP-1 activation because JBD, a dominant negative mutant of JNK interacting protein (JIP) that blocks signal transduction from MEKK1 to AP-1, did not repress MEKK1-activating PSA transcription. However, the activation was abolished by a dominant negative mutant of IkBa, of which both ser32 and 36 were substituted by alanine. This result suggests that MEKK1 activates the PSA enhancer through NF-kB activation. This conclusion was confirmed by two other observations. First, TNFa, a stimulus that leads to NF-kB activation, activates the PSA enhancer. Second, overexpression of the p65 subunit of NF-kB was sufficient to activate the PSA enhancer. The IkBa mutant abrogates the activation by both TNFa and overexpression of p65. By DNA footprinting analysis, we identified four NF-kB binding sites in the PSA enhancer. We also observed that androgen–independent LAPC4 tumor cells have a higher constitutive activity of NF-KB than the LAPC4 androgen–dependent tumors, consistent with other reports showing that androgen unresponsive cell lines, DU145 and PC3, have a higher constitutive activity of NF-kB than the androgen responsive LNCaP cell line.

These studies support a potential role for the NF-kB pathway in the transition of some prostate cancers to an androgen-independent state. We are now in the process of directly testing this hypothesis by engineering constitutive high NF-kB activity in androgen-dependent prostate cancer models.

Research Progress Part II, BRCA1 Inhibits the Transcription Activity of Androgen Receptor by Recruiting HDAC Complex

Germ-line mutations of the BRCA1 gene account for half of hereditary breast cancers and confer increased risk for ovarian and prostate cancers. This gene encodes an 1863-amino acid protein that has been implicated in the regulation of cell proliferation, cell cycle progression, apoptosis, DNA repair and recombination. The expression of the BRCA1 gene was shown to be down-regulated in breast cancer progression. It was also demonstrated that the BRCA1 gene product inhibits estrogen receptor transcription activity. Based on the similarity between prostate and breast cancer progression, and the conservation between androgen and estrogen receptor, we hypothesized that the BRCA1 protein is involved in the progression of prostate cancer.

By transient transfection experiment, we demonstrated that the BRCA1 protein inhibits androgen receptor transcription activity. Western blot analysis indicates a decreased expression of the BRCA1 protein in LAPC4 androgen-independent tumors than in LAPC4 androgen-dependent tumors. Because the transcription activity of androgen receptor is believed to be important for prostate cancer progression, these results support our hypothesis that BRCA1 is involved in prostate cancer progression. The inhibition of BRCA1 protein on androgen receptor can be released by the addition of TSA, a specific inhibitor of histone deacetylase (HDAC). We also detected a physical interaction between the BRCA1 protein and androgen receptor. These results suggest that the BRCA1 protein inhibits the transcription activity of androgen receptor through HDAC, consistent with the finding that the BRCT repeats at the C -termini of the BRCA1 protein interacts with the HDAC complex. To confirm our conclusion, we generated a BRCA1 expression plasmid that contains only the BRCT repeats (named BRCT), and another plasmid that has a cytosine insertion at 1853 position (named Cinsert). The C insertion frame-shifts the translation of the BRCT repeats and is a common mutation found in hereditary breast, ovary and prostate cancer patients. Both the BRCT and the Cinsert abolish the inhibition of the BRCA1 protein on the transcription activity of androgen receptor. We are now determining the interactions among androgen receptor, the BRCA1 protein, and the HDAC complex, and the disruption of these interaction by the BRCT.